Title page

Pancreatic Adenocarcinoma With Strong Expression of Interleukin-13 Receptor α2 Shows a Poor Response to Gemcitabine-Based Chemotherapy

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Running title: PDAC With Strong Expression of IL-13Rα2

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Abstract

Objectives: Pancreatic ductal adenocarcinoma with strong expression of interleukin-13 receptor α2 (IL-13Rα2) was associated with poor prognosis and gemcitabine resistance in an orthotopic mouse model. We evaluated the influence of IL-13Rα2 expression in the endoscopic ultrasound-fine needle aspiration (EUS-FNA) specimen.

Methods: We included patients with pancreatic ductal adenocarcinoma, as diagnosed by EUS-FNA, who received gemcitabine-based chemotherapy. Tumor expression of IL-13Rα2 was assessed by immunohistochemistry and classified using a three scale [negative, weak or strong] in a blinded fashion. The effect of gemcitabine-based chemotherapy was assessed by tumor reduction rate by computed tomography after 3 months.

Results: A total of 95 patients were enrolled, and 63 and 32 cases were determined with strong and weak/negative expression of IL-13Rα2. The IL-13Rα2-strong group showed significantly poorer progression-free and overall survival rates than weak/negative group (P = 0.0191 and P = 0.0062, respectively). Strong expression of IL-13Rα2 was associated with progression factor after 3 months of the first gemcitabine-based chemotherapy (odds ratio, 13.72, P = 0.0143).

Conclusions: Pancreatic ductal adenocarcinoma with strong expression of IL-13Rα2 in EUS-FNA specimens showed poor prognosis and poor response to gemcitabine-based chemotherapy.

Key Words: pancreatic ductal adenocarcinoma, EUS-FNA, IL-13Rα2, gemcitabine resistance, prognosis

TEXT

INTRODUCTION

Interleukin-13 receptor α2 (IL-13Rα2) is a single-transmembrane protein consisting of 380 amino acids. It is a subunit of the IL-13 receptor family. Interleukin-13 receptor α2 has high affinity for IL-13, and IL-13 binding has complex internal effects and functions.[1] Interleukin-13 receptor α2, previously considered a decoy receptor, is involved in intracellular signaling.[2] Interleukin-13 receptor α2 reportedly promotes metastasis of human breast cancer, and decreased expression of IL-13Rα2 suppresses metastasis.[3] It has served as a prognostic and drug resistance factor in glioblastoma, renal cell carcinoma, and gastric cancer.[4-7] In glioblastoma, immunosuppressive genes were highly expressed in IL-13Rα2 positive tumors and immunosuppressive genes associated with IL-13Rα2 may play a role in tumor progression.[6] Interleukin-13 receptor α2-mediated resistance to sunitinib is caused by the inhibition of sunitinib-induced apoptosis without increasing renal cell carcinoma microvasculature.[7] We showed that strong expression of IL-13Rα2 is related to a poor prognosis due to the promotion of metastasis in an orthotopic mouse model of pancreatic cancer. In the study, IL-13 activates the MAPK pathway, especially ERK1/2, via IL-13Rα2 and increases the levels of matrix metalloproteinases; these mechanisms are implicated in the metastasis of pancreatic cancer.[8] Because the expression of this receptor is largely specific for cancer cells and noncancerous cells do not express it, IL-13Rα2 would be a diagnostic and therapeutic target for cancer.

The five-year survival rate of pancreatic ductal adenocarcinoma (PDAC) is only 2% despite multimodal treatment.[9] Poor prognostic factors in PDAC include resistance to chemotherapy and early invasive or metastatic disease. Gemcitabine is a key drug for PDAC, and numerous trials of gemcitabine-based double/triple therapies have been performed.[10-12] A poor response to gemcitabine may shorten survival, so early identification of gemcitabine resistant PDAC cases is important. In a mouse model, the administration of gemcitabine and an *IL-13Rα2*-target immunotoxin (a fusion protein of *IL-13* and *Pseudomonas* exotoxin) exerted a synergistic effect on PDAC, significantly

reducing tumor volume.[13] We found an inverse correlation between IL-13Rα2 expression in surgical specimens of PDAC and prognosis. The role of endoscopic ultrasound-fine needle aspiration (EUS-FNA) has shifted from diagnosis to tissue acquisition for histological, immunohistochemical, and molecular analyses. Because the heterogeneity of IL-13Rα2 expression in pancreatic cancer is known to be minimal, it is worth evaluating the predictivity of IL-13Rα2 expression using biopsy specimen.[14] If EUS-FNA specimens at the time of diagnosis before treatment enable evaluation of IL-13Rα2 expression similarly to surgical specimens, initial treatment selection would be possible as well as prognostic prediction.

We analyzed the expression of IL-13Rα2 in EUS-FNA specimens obtained at the time of diagnosis and examined the relationship between IL-13Rα2 expression and the tumor reduction rate and prognosis of patients with PDAC who received systemic chemotherapy.

MATERIALS AND METHODS

We analyzed patients with PDAC who received systemic chemotherapy for initial treatment. A flowchart of the case selection process is shown in Figure 1. Briefly, between April 2014 and April 2019, 231 patients were histologically diagnosed with pancreatic cancer by EUS-FNA at Juntendo University Hospital, Tokyo, Japan. First, 18 patients with intraductal papillary mucinous carcinoma, neuroendocrine carcinoma, or acinar cell carcinoma, and 8 with samples unsuitable for immunohistochemistry (IHC), were excluded; 22 patients with best supportive care not receiving chemotherapy and 51 patients who underwent surgery were also excluded. Next, 132 who received chemotherapy were identified. Finally, 95 patients were included in the analysis: received gemcitabine-based chemotherapy (G-CTX) as an initial treatment but could not continue chemotherapy for >3 months, excluding 10 who started fluorinated pyrimidine-based chemotherapy (Fig. 1).

Clinical data were extracted from the electronic medical records and the relationship of IL-13Rα2 expression with the tumor reduction rate and prognosis was examined. Overall survival (OS) and

progression-free survival (PFS) were analyzed from initiation of chemotherapy or chemoradiotherapy to death, and from treatment to the date of local progression >20% or distant metastasis based on the RECIST guidelines.[15] In addition, the primary tumor reduction rate was compared between the high and low IL-13Rα2 expression groups. Tumors were staged according to the guidelines of the Union for International Cancer Control and the American Joint Committee on Cancer.[16]

IHC

Four-micron-thick PDAC tissue sections were prepared on poly-L-lysine coated glass slides. Hematoxylin and eosin staining and IHC for IL-13Rα2 was performed as described previously.[17] Briefly, IL-13Rα2 expression in PDAC and normal tissues were determined using a goat polyclonal antibody against IL-13Rα2 (R&D Systems, Minneapolis, Minn). The sections were deparaffinized, dehydrated in 100%, 75%, and 50% alcohol and treated with an antigen unmasking reagent to unmask IL-13Rα2 protein. Autofluorescence in paraffin tissue sections was minimized by incubation with 1% sodium borohydride solution for 2 h and incubated in blocking buffer consisting of 5% rabbit serum and 1% biotin-free bovine serum albumin in 18× phosphate-buffered saline for 2 h. The paraffin tissue sections were immunostained with 0.5 μg/mL IL-13Rα2 antibody overnight at 4°C, washed twice with 1× phosphate-buffered saline, and incubated with a biotinylated rabbit anti-goat antibody (Histofine Simple Stain MAX PO; 414161F; Nichirei Biosciences, Inc., Tokyo, Japan). The samples were incubated with iminoctadine or isotype control goat IgG (negative control).

With hematoxylin and eosin specimen, PDAC histology was classified into wel, mod, or por, according to the WHO classification.[18] The quantity of the PDAC in each specimen was also recorded and classified into abundant, in which cancer cells occupied >3 × 3 mm area; moderate, >1 × 1 mm but <3 × 3 mm area; or scarce, <1 × 1 mm area of EUS-FNA specimens.

For evaluation of IL-13Rα2 expression, each case was classified into strong, weak or negative expression, based on the intensity of staining. The intensity of IL-13Rα2 expression was defined as follows, strong for cases in which hematoxylin staining was not visible (Fig. 2A, arrow), weak for

cases in which both hematoxylin and DAB stain was visible (Fig. 2B, arrow), and negative for cases in which no DAB staining was recognized (Fig. 2C, arrow). For the further comparison and statistical analyses, cases were divided into IL-13Rα2-strong and IL-13Rα2-negative/weak (Fig. 2). Interleukin-13 receptor α2 expression was evaluated independently by three researchers (K.T., T.F., and Y.F.), one of whom was a pathologist, in a blinded fashion. The IL-13Rα2 IHC evaluation was consistent among most specimens. If different staining intensities were observed within the same tumor, the largest expression area was recorded. Evaluation on which all three researchers agreed, or those agreed upon by two researchers including the certified pathologist, were used as the final evaluation. Otherwise, final evaluation was determined after discussion among the three researchers.

Clinical Database for Pancreatic Cancer

For 95 patients who could be stained with IL-13Rα2 and were suitable for continuous gemcitabine-based chemotherapy, a clinical database was created from the electronic medical records. Patient characteristics (age, sex, performance status [PS], body mass index, and the modified Glasgow prognostic score [mGPS]), tumor markers (carbohydrate antigen 19-9 [CA 19-9], DUPAN-2, and SPAN-1), computed tomography (CT) findings (tumor size, location, vessel invasion, stage), pathological diagnosis by EUS-FNA (differentiation and histological type), treatment (surgery, chemotherapy, or chemoradiotherapy), OS, and PFS were extracted. The mGPS incorporates the C-reactive protein (CRP) and albumin levels, which reflect systemic inflammation, and is a validated instrument that stratifies patients into three groups according to prognosis.[19] The standard values were age >65 years, PS of 2; body mass index >18.5 kg/m^2, mGPS of 2, tumor markers above the median values and a tumor size of >35 mm.

Statistical Analysis

The chi- squared or Fisher's exact test and Mann-Whitney U test were used to analyze the data, as appropriate. Pairwise comparisons between groups were conducted using the Wilcoxon signed-rank test. Survival curves were generated by the Kaplan-Meier method and compared by the log-rank test.

Multivariate analysis (logistic regression analysis/Cox's proportional hazards model) of all clinical parameters and chemotherapy resistance/prognostic factors was performed. The results are expressed as hazard ratios (HRs), 95% confidence intervals (CIs), and *P* values (where $P < 0.05$ indicated significance). Statistical analysis was performed using BellCurve for Excel statistical software (Microsoft Corp., Redmond, Wash).

RESULTS

Patient Characteristics and IL-13Rα2 Expression

The clinicopathological characteristics of the 95 patients are listed in Table 1. The median age was 69 (interquartile range, 64–75) years, and the female ratio was 42.1 %. Histological diagnosis was adenocarcinoma for all and consisted of 13/49/31 cases of well/moderately/poorly differentiated carcinoma, respectively. The quantity of the cancer cells in EUS-FNA specimens were abundant, moderate, and scarce in 66/21/6 cases, respectively. The tumor location was the head in 55.8 % of cases and the body or tail in 44.2 %; 1, 6, 39, and 49 cases were Union for International Cancer Control stage I-IV, respectively, at the time of treatment. The median CA 19-9 level was 252 (interquartile range, 62.5–1361) U/mL. There were no significant differences in characteristics between the IL-13Rα2-high and -low groups (age, $P = 0.8$; sex, $P = 0.8$; tumor location, $P = 0.5$; Union for International Cancer Control stage, $P = 0.7$; CA 19-9, $P = 0.3$; treatment, $P = 0.9$). Chemotherapy and chemoradiotherapy were conducted in 83.2% and 16.8% of cases, respectively. The chemotherapy regimen was gemcitabine plus nab-paclitaxel in 82.1% of cases, only gemcitabine in 11.6% and gemcitabine plus S-1 in 6.3%.

We found that IL-13Ra2 expression was consistent throughout each specimen in most cases (89/95, 93.7%), irrespective of the slight histological changes of the PDAC, ie, well-differentiated PDAC components and poorly differentiated components in one specimen showed same IHC intensity in 93.7% of the cases. Interleukin-13 receptor α2 expression was determined strong in 64 cases (67.4%)

and weak/negative in 31 cases (32.6%) (Fig. 2). At initial evaluation of the IL-13Rα2 score, the three researchers scored 10 cases (10.5%) differently, among which 5 cases (50.0%) were rich in cytoplasmic mucus and 6 (60.0%) showed heterogeneous staining.

Association of IL-13Rα2 Expression With Chemotherapy Efficacy

The changes in tumor size after 3 months of chemotherapy are shown as Waterfall plots (Fig. 3). The IL-13Rα2-strong group had a significantly higher ratio of progressive disease (PD) compared to the IL-13Rα2-weak/negative group (35.9% vs 3.2%, $P < 0.001$, respectively). The IL-13Rα2-strong group showed significantly shorter median OS and PFS than the IL-13Rα2-weak/negative group (OS, 13.0 vs 22.0 months; HR, 1.99; 95% CI, 1.2–3.3; $P = 0.0062$) (PFS; 6.0 vs 11.0 months; HR, 1.68; 95% CI, 1.1–2.7, $P = 0.0191$) (Fig. 4). There was no significant difference between the IL-13Rα2-strong and -weak/negative groups in the frequency of adverse events due to chemotherapy, including neutropenia. There was also no group difference in the dose reduction ratio (59.5% vs 62.5%, $P = 0.7$).

The First Efficacy of Gemcitabine-Based Chemotherapy Between IL-13Rα2-high Group and -Low Group

The efficacy of 3 months of G-CTX was assessed by CT. The IL-13Rα2-strong group had a significantly higher rate of PD rate than the IL-13Rα2-weak/negative group (35.9% vs 3.2%, $P < 0.001$) (Table 2A). Odd ratios (ORs) and 95% CIs from logistic regressions of factors associated with PD after 3 months G-CTX (Table 2B). Compared to other factors (PS, mGPS, CA 19-9 and chemotherapy dose reduction), IL-13Rα2 was associated with PD risk on the first efficacy (IL-13Rα2; ORs, 13.72 (95% CI, 1.68-111.5, $P = 0.0143$)).

Univariate and Multivariate Analyses of Prognostic Factors for Chemotherapy Efficacy and OS

In univariate analyses, PS of 0-1 or 2 ($P < 0.01$), mGPS of 0-1 or 2 ($P < 0.01$), tumor size <32 or ≥32 mm ($P = 0.02$), surgery ($P < 0.01$), radiation ($P < 0.01$), and tumoral IL-13Rα2 expression ($P <$

0.01) were associated with OS (Table 3). In multivariate analysis, no surgery, no radiation, and strong IL-13Rα2 expression of the tumor were associated with a poorer prognosis (HR, 0.31; 95% CI, 0.12–0.81; *P* = 0.02; HR, 0.35; 95% CI, 0.17–0.73; *P* = 0.01; and HR, 1.81; 95% CI, 1.02–3.22; *P* = 0.04, respectively) (Table 3).

DISCUSSION

Endoscopic ultrasound-fine needle aspiration plays roles not only in histological diagnosis, but also in the evaluation of tumor status (which informs treatment selection). In pancreatic cancer, microsatellite instability status is routinely determined to assess the suitability of pembrolizumab.[20] Commercial oncogene panels typically use EUS-FNA specimens.[21] We identified an inverse relationship between tumoral IL-13Rα2 expression and PDAC prognosis through analysis of a large sample of surgical specimens.[14] Here, we focused on the ability of tumoral IL-13Rα2 expression in EUS-FNA specimens to predict prognosis and/or chemotherapy efficacy. Predicting prognosis or chemotherapy efficacy using EUS-FNA rather than surgical specimens has several advantages. Considering the low rate (20%) of surgery for PDAC, EUS-FNA specimens, which can be obtained in almost 100% of cases, may be the only material suitable for pathological and genetic evaluation. In addition, knowledge of chemoresistance, particularly to gemcitabine, at the time of diagnosis facilitates selection of an appropriate chemotherapy regimen.

Interleukin-13 receptor α2 expression revealed by IHC of EUS-FNA specimens was predictive of OS and PFS (Figure 4) and the response to chemotherapy. The relationship between tumoral IL-13Rα2 expression and the first response to chemotherapy was evidenced by the rates of reduction in tumor volume (Figure 3 and Table 2-a). We previously reported a poorer response to chemotherapy of *IL-13Rα2*-high compared to *IL-13Rα2*-low PDAC in a mouse model.[13] Moreover, combination therapy with gemcitabine and an *IL-13Rα2*-targeting immunotoxin exerted a synergistic effect on mouse PDAC. In this study, PS (*P* < 0.01), mGPS (*P* < 0.01), tumor size (*P* = 0.02) and surgery (*P* < 0.01)

were strongly associated with prognosis, as reported by other studies.[22-24] Radiotherapy was related to a better prognosis, likely because radiotherapy is typically performed for stage II and III PDAC. In a multivariate analysis, the presence of surgery (P = 0.02), the presence of radiotherapy (P = 0.01), and negative tumoral IL-13Rα2 expression (P = 0.04) contribute better prognosis significantly, suggesting that evaluation of EUS-FNA specimens for tumoral IL-13Rα2 expression at the time of diagnosis enables prediction of prognosis.

Although PDAC is a histologically heterogeneous tumor in which well to poorly differentiated components, even anaplastic components are often mixed in one case, our previous study has shown that IL-13Ra2 IHC shows almost consistent result.[14] In this study, 93.7% of the studied cases showed consistent IHC results. Regarding the remaining cases (6.3%) which showed different IHC results area by area, we think there may be two factors which influence on the IL-13Ra2 expression; (i) PDAC at the invasive front or at the internal site, and (ii) the molecular progression, such as epithelial-mesenchymal transition. In our previous study with surgical cases, IL-13Rα2 expression was higher at peripheral site of the tumor compared to the internal site in some cases, suggesting biological or molecular tumor progression may affect IL13Ra2 expression (data not shown). Further studies are needed to determine the cause of IL-13Rα2 heterogeneity, although which is rare in PDAC.

The limitation of the present study is that some difficulty exists in IL-13Ra2 IHC evaluation in PDAC cases with abundant intracytoplasmic mucin. In this retrospective study, the initial agreement rate among the three researchers on the evaluation of IL-13Rα2 IHC was 89.5% and among the 10.5% of initial disagreement cases were due to abundant intracytoplasmic mucus. In such cases, looking for tumor cells with mucin-free or mucin-less and evaluating such tumor cells are important. When the staining was heterogeneous by tumor area, the IHC score for more than 50% of the tumor cells were adopted for the case. As for another limitation for this research, we showed IL-13Rα2 high group has worse survival without discussing second line chemotherapy. The mechanism by which IL-13Rα2 induces gemcitabine resistance in pancreatic cancer is unknown and will be investigated by genetic

analysis as future issues.

Our analysis suggested that strong expression of IL-13Rα2 may reduce the effectiveness of G-CTX and be poorer prognosis. Because EUS-FNA specimens was good enough to evaluate IL-13Rα2 characteristics same as former study[25], we could facilitate selection of the most suitable chemotherapeutic agent in referring to EUS-FNA specimens obtained at the time of diagnosis.

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Abbreviations: CA 19-9, carbohydrate antigen 19-9; EUS-FNA, endoscopic ultrasound-fine needle aspiration; FFPE, formalin fixed paraffin embedded; G-CTX, gemcitabine-based chemotherapy; IL-13Rα2, Interleukin-13 Receptorα2; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression free survival; PS, performance status.

Author Contributions: K.T., To.F., and H.I. conceptualized the study. M.U., Ta.F., S.T., and Y.T. worked on data curation. A.S. performed formal analysis. K.T. and To.F. worked on funding acquisition. K.I. participated in the investigation. S.I. worked on methodology. Y.F. worked on project administration. K.T. worked on software. T.Y. and A.N. supervised. Y.F. worked on validation. Y.F. worked on visualization. K.T. wrote the original draft. Y.F., To.F., and H.I. did the review and editing.

Disclosure Statement

This study was approved by the Ethics Committee of Juntendo University (No. 2021084) and conducted according to the Ethical Guidelines for Human Genome / Gene Research of the Japanese Government and the Helsinki Declaration. All authors have read and agreed to the revised version of the manuscript.

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FIGURE LEGENDS

FIGURE 1. Patient flowchart. ACC, acinar cell carcinoma; BSC, best supportive care; EUS-FNA, endoscopic ultrasound-fine needle aspiration; IPMC, intraductal papillary mucinous carcinoma; NEC, neuroendocrine carcinoma. *Withdraw - 27 patients who had difficulty continuing chemotherapy for more than 3 months.

FIGURE 2. Immunohistochemical evaluation IL-13Rα2 expression (representative cases) based on the intensity of staining, each case was classified into strong (Score 2), weak (Score 1), and negative (Score 0) expression. The intensity of IL-13Rα2 expression was defined as follows, strong, for cases in which hematoxylin staining was not visible (A, arrow), weak, for cases in which both hematoxylin and DAB stain was visible (B, arrow), and negative, for cases in which no DAB staining was recognized (C, arrow). For the further comparison and statistical analyses, cases were divided into IL-13R2-strong and IL-13R2-negative/weak. The evaluation of IL-13Rα2 expression was 64 cases of strong, 29 cases of weak and 2 cases of negative. Hence, 67.4% and 32.6% of these cases were categorized into the IL-13Rα2-strong and -negative/weak groups as the final evaluation, respectively.

FIGURE 3. Association between tumor reduction and IL-13Rα2 expression after 3 months of chemotherapy (waterfall plot). The IL-13Rα2-strong group had a high ratio of progressive disease (PD) compared to the IL-13Rα2-weak/negative group (35.9 % vs 3.2 %, $P < 0.001$, respectively).

FIGURE 4. Relationship between IL-13Rα2 expression and OS/PFS. IL-13Rα2-strong group significantly shortened OS/PFS compared to the IL-13Rα2-weak/negative group (median OS, 13.0 mo vs 22.0 mo, $P = 0.0062$ and median PFS, 6.0 mo vs 11.0 mo, $P = 0.0191$).

Table 1

TABLE 1. Clinicopathologic Characteristics of the Patients (n = 95)

Parameters	
Age, median (IQR), y	69 (64-75)
Sex, male/female, n (%)	
Male	55 (57.9)
Female	40 (42.1)
Histologic diagnosis, n (%)	
Adenocarcinoma	95 (100)
Histological diagnosis, n (%)	
Well	13 (13.7)
Moderately	49 (51.6)
Poorly	31 (32.6)
NA	2 (2.1)
Quantity of the cancer cells	
Abundant	66 (69.5)
Moderate	21 (22.1)
Scarce	6 (6.3)
NA	2 (2.1)
Tumor Location, n (%)	
Head	53 (55.8)
Body/tail	42 (44.2)
UICC clinical stage, n (%)	
I	1 (1.1)
II	6 (6.3)
III	39 (41.1)
IV	49 (51.5)
CA 19-9, U/mL*	252 (62.5–1361)
Treatment, n (%)	
Chemotherapy	79 (83.2)
Chemoradiotherapy	16 (16.8)
Regimen, n (%)	
Gemcitabine plus nab-paclitaxel	78 (82.1)
Gemcitabine	11 (11.6)
Gemcitabine plus S-1	6 (6.3)

CA 19-9 indicates carbohydrate antigen 19-9; IQR, interquartile range; NA, not applicable.

Table 2

TABLE 2. Progressive Disease After 3 Months of Gemcitabine-Based Chemotherapy

A. Progressive disease ratio in the IL-13Rα2-strong group receiving gemcitabine-based chemotherapy after 3 mo

	IL-13Rα2-Weak/Negative, n (%)	IL-13Rα2-Strong, n (%)	*P*
n	31	64	
PD	1 (3.2)	23 (35.9)	<0.001
SD/PR/CR	30 (96.8)	41 (64.1)	

B. Factors associated with progressive disease after 3 mo gemcitabine-based chemotherapy

Factors	OR (95% CI)	*P*
PS ≥2	4.52 (0.72-28.27)	0.106
mGPS ≥2	2.65 (0.61-11.51)	0.192
CA 19-9 <252 U/mL	0.81 (0.28-2.29)	0.696
Chemotherapy dose reduction	1.48 (0.51-4.28)	0.464
IL-13Rα2	13.72 (1.68-111.57)	**0.014**

IL-13Rα2 was associated with PD risk on the first efficacy. Bold values are statistically significant.

CR indicates complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 3

TABLE 3. Univariate and Multivariate Analysis of Prognostic Factors Associated With Overall Survival

			Univariate Analysis	Multivariate Analysis	
Factors (Median)	**n**	**Median Survival, mo**	***P***	**Hazard Ratio (95% CI)**	***P***
Age, y			0.43		
<65	27	16			
≥65	68	15			
Sex			0.84		
Female	40	14			
Male	55	17			
PS			**<0.01**	1.32 (0.50–3.49)	0.56
0–1	89	17			
2	6	4			
Body mass index, kg/m^2			0.13		
<18.5	32	14			
≥18.5	63	19			
mGPS			**<0.01**	1.82 (0.77–4.32)	0.17
0–1	86	17			
2	9	9			
CA 19-9, U/mL (252)			0.65		
<252	48	14			
≥252	47	17			
DUPAN2, U/mL (150)			0.88		
<150	35	17			
≥150	31	17			
SPAN1, U/mL (110)			0.92		
<110	45	14			
≥110	43	17			
Tumor size, mm			**0.02**	0.79 (0.47–1.32)	0.37
<32	44	20			
≥32	51	12			
Location			0.79		
Head	53	17			
Body/tail	42	15			
PV invasion			0.31		

Absent	79	15			
Present	16	21			
Artery invasion			0.50		
Absent	44	14			
Present	51	17			
Differentiation			0.35		
Well/moderately	30	19			
Poorly	25	13			
Surgery			**<0.01**	0.31 (0.12–0.81)	**0.02**
Absent	82	14			
Present	13	NR			
Radiation			**<0.01**	0.35 (0.17–0.73)	**0.01**
Absent	79	14			
Present	16	31			
IL-13Rα2			**<0.01**	1.81 (1.02–3.22)	**0.04**
Weak/negative	31	22			
Strong	64	13			

In univariate analyses, PS of 0 - 1 or 2 ($P < 0.01$), mGPS of 0-1 or 2 ($P < 0.01$), tumor size <32 or ≥32 mm ($P = 0.02$), surgery, ($P < 0.01$), radiation ($P < 0.01$), and tumoral IL-13Rα2 expression ($P < 0.01$) were associated with OS. In multivariate analysis, no surgery, no radiation, and strong IL-13Rα2 expression of the tumor were associated with a poorer prognosis (HRs, 0.31, 95% CI, 0.12–0.81, $P = 0.02$; HRs, 0.35, 95% CI, 0.17–0.73, $P = 0.01$; and HRs, 1.81, 95% CI, 1.02–3.22, $P = 0.04$, respectively). Bold values are statistically significant.

CA 19-9 indicates carbohydrate antigen 19-9; mGPS, modified Glasgow prognostic score; NR, not reached to median; PS, performance status; PV, portal vein.

Figure 1

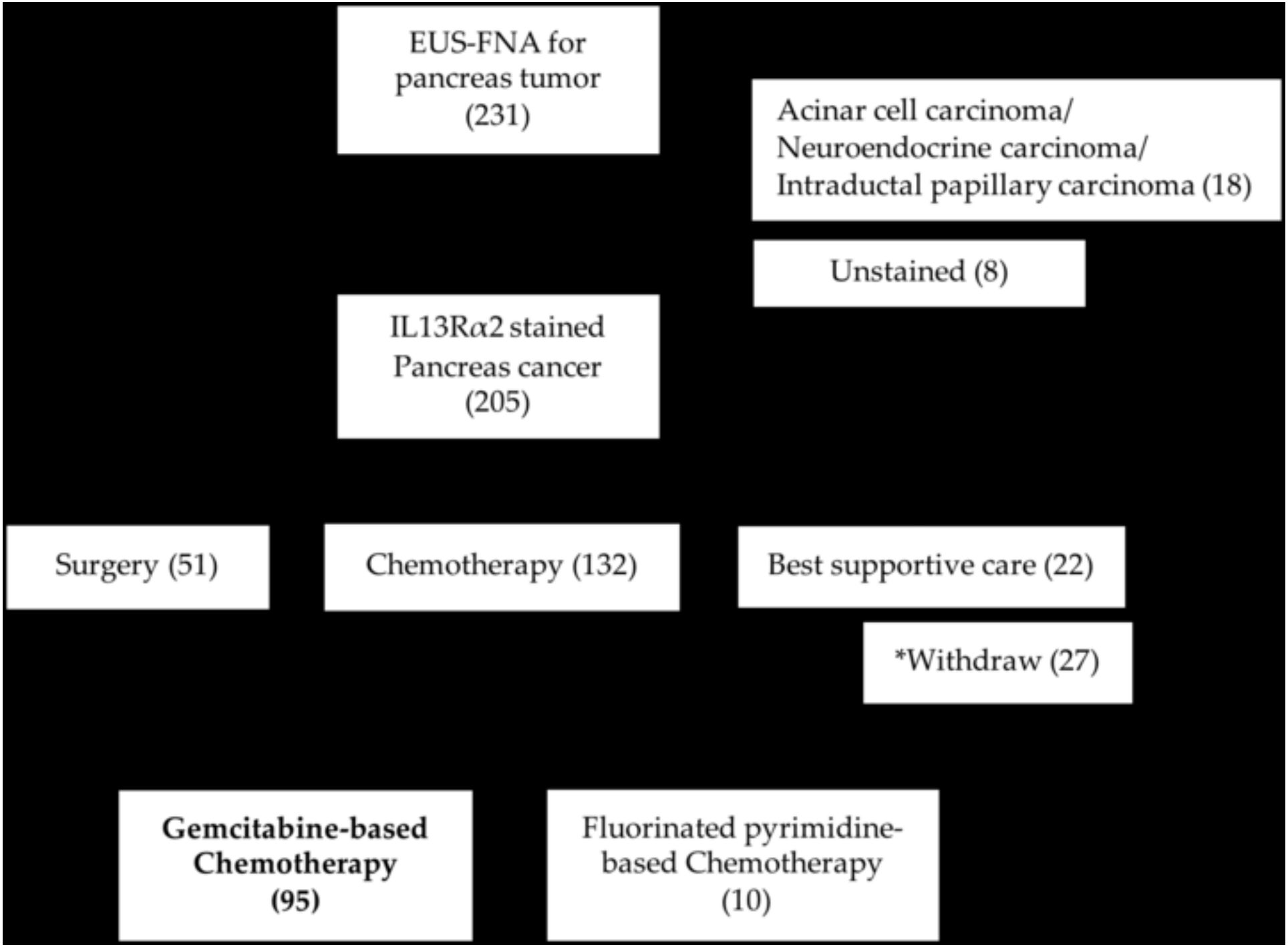

Figure 2

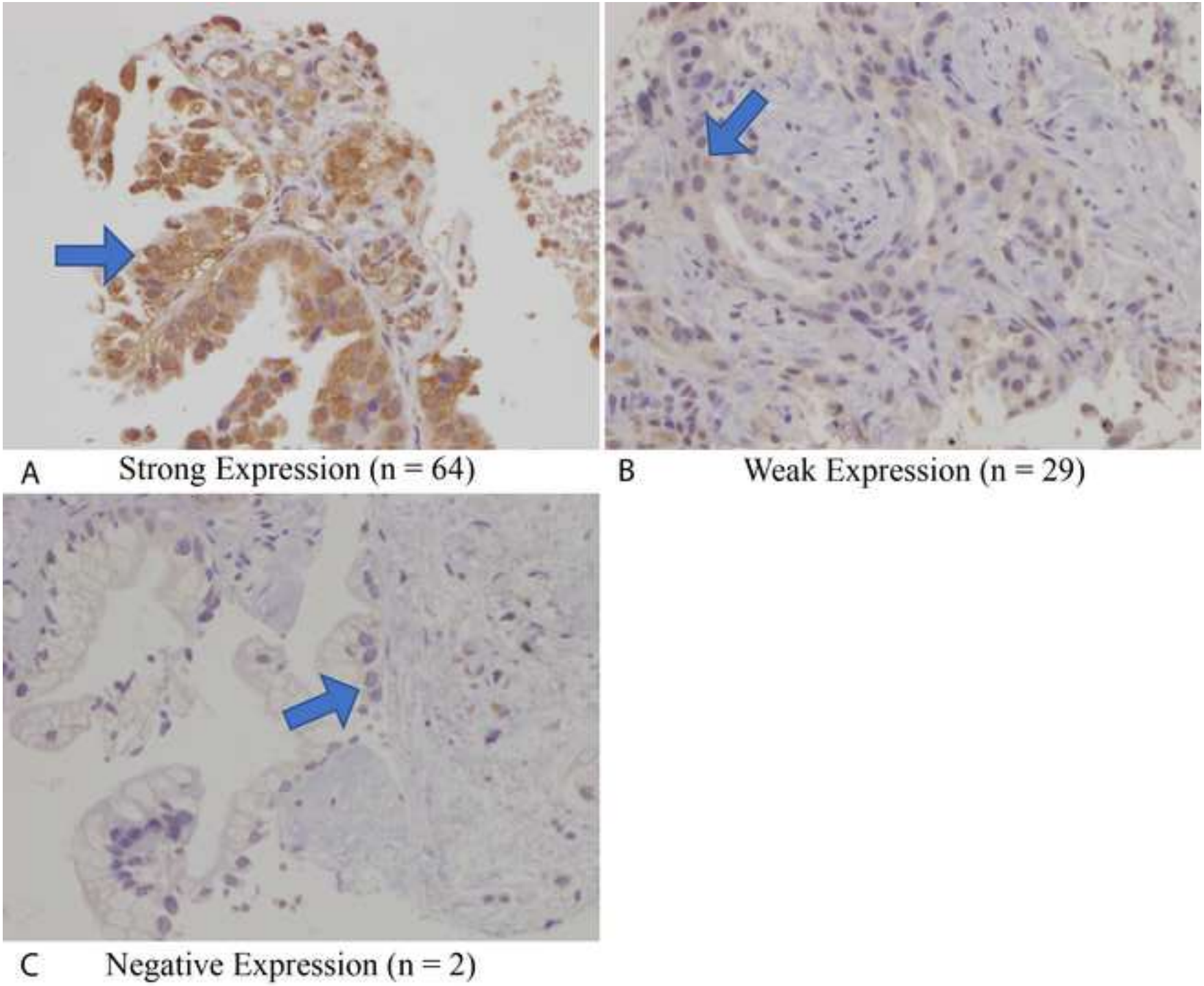

Figure 3

Click here to access/download;Figure;Figure 3.tif

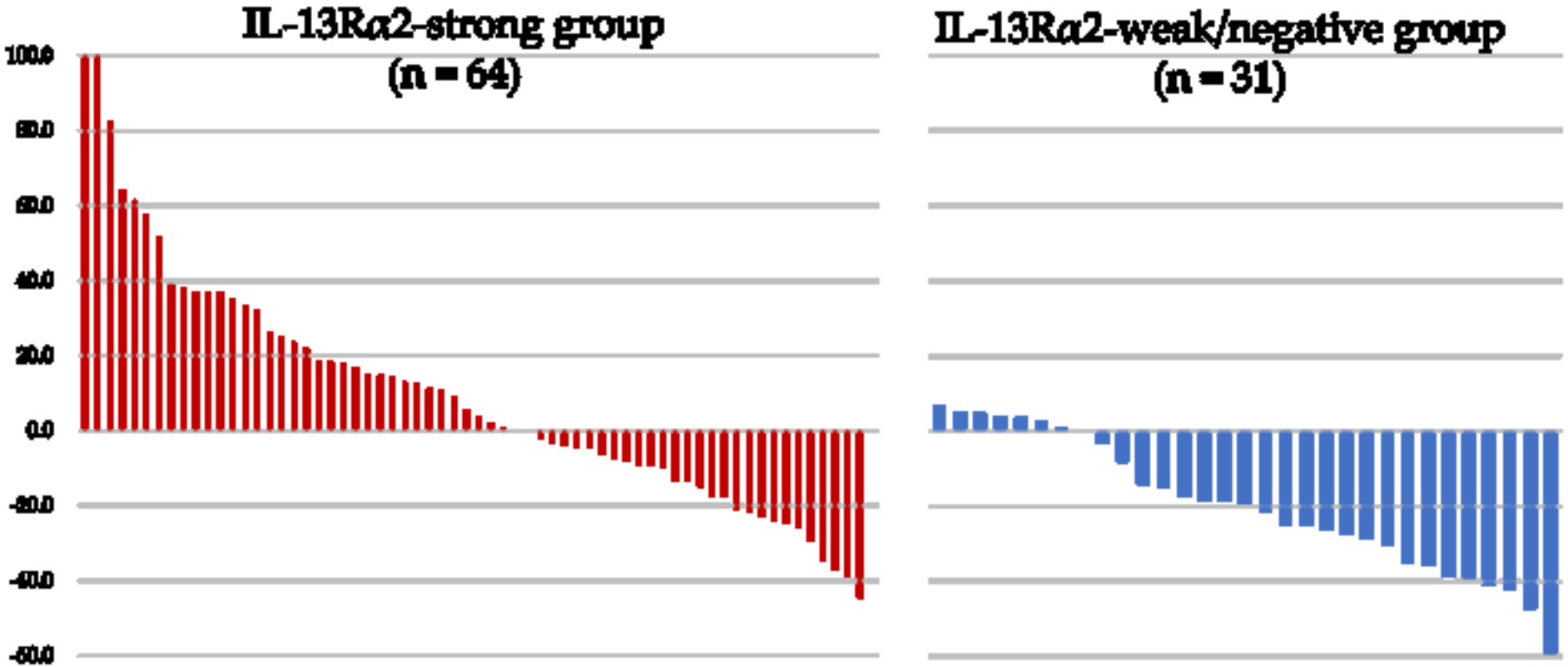

Figure 4